Electronic Supplementary Material (ESI) for Analyst.

Bacterial Detection With Amphiphilic Carbon Dots

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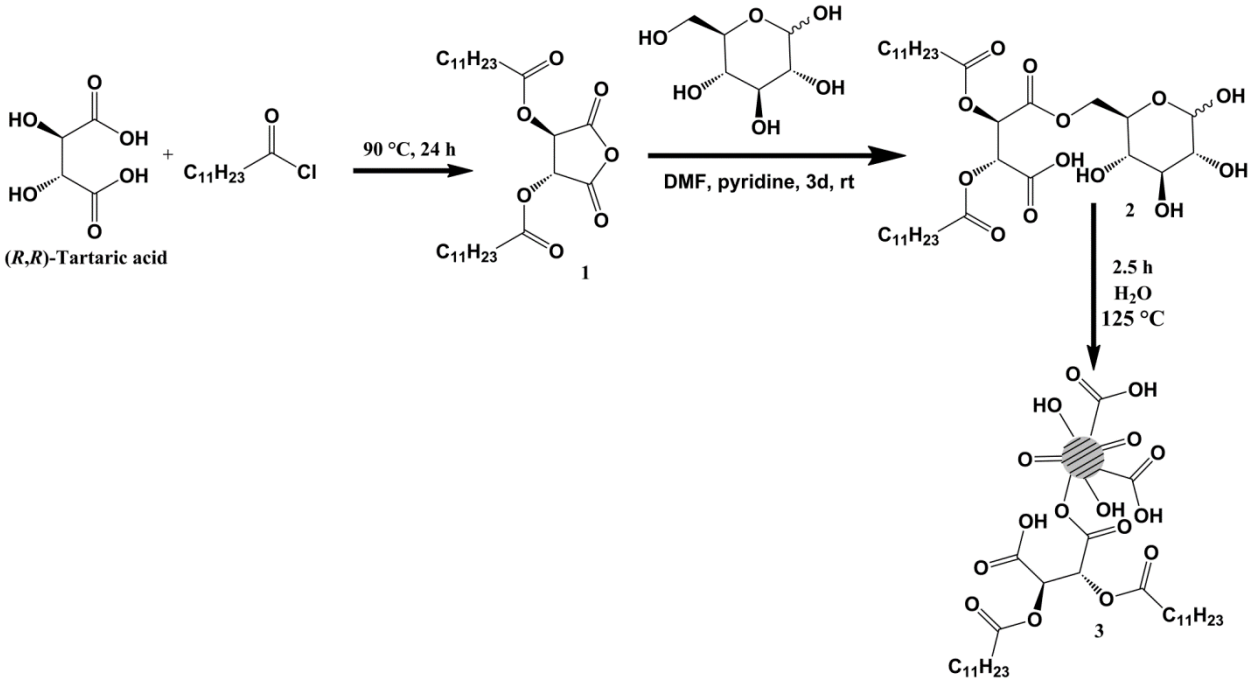

Syntheses and spectroscopic characterisation of compound 1-3

Syntheses and spectroscopic characterisation of (3*R*,4*R*)-2,5-Dioxotetrahydrofuran-3,4-diyl didodecanoate (compound 1)

In a 500 mL round bottom flask equipped with a magnetic stirrer bar and an air bubbler 166 mL of lauroyl chloride (0.7 mol) and 30 g of finely powdered *L*-tartaric acid (0.2 mol) were added. The reaction was continued at 90 °C for 24 h and then cooled to room temperature. To remove lauric acid and excess lauroyl chloride, the crude mixture was dissolved in a minimum amount of *n*-hexane and kept at room temperature for 12 h. The product was precipitated in *n*-hexane which was

then collected through filtration, washed thoroughly with *n*-hexane, and dried under vacuum. to obtain 89 g of compound **1** (90%) as white powder.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.67 (s, 2H), 2.48 (t, *J* = 7.5 Hz, 4H), 1.68 (m, 4H), 1.49 – 1.09 (m, 32H), 0.90 (t, *J* = 7.1 Hz, 6H) ppm.
^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.60, 163.48, 72.05, 33.30, 31.87, 29.55, 29.54, 29.36, 29.29, 29.12, 28.85, 24.51, 22.64, 14.04 ppm.

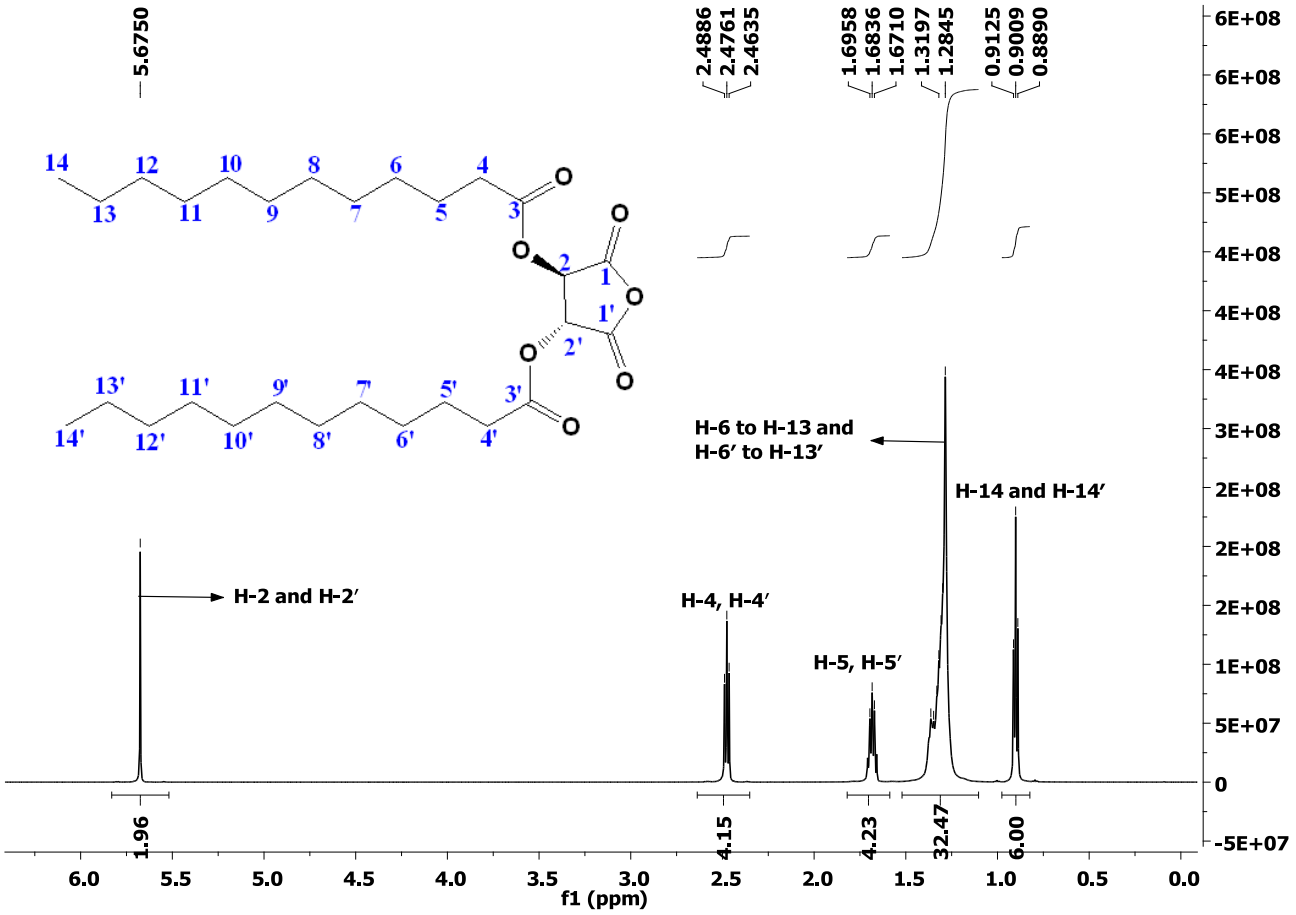

Figure 1, SI: ^{1}H NMR spectrum of compund 1 in $CDCl_3$ recorded at 298 °K.

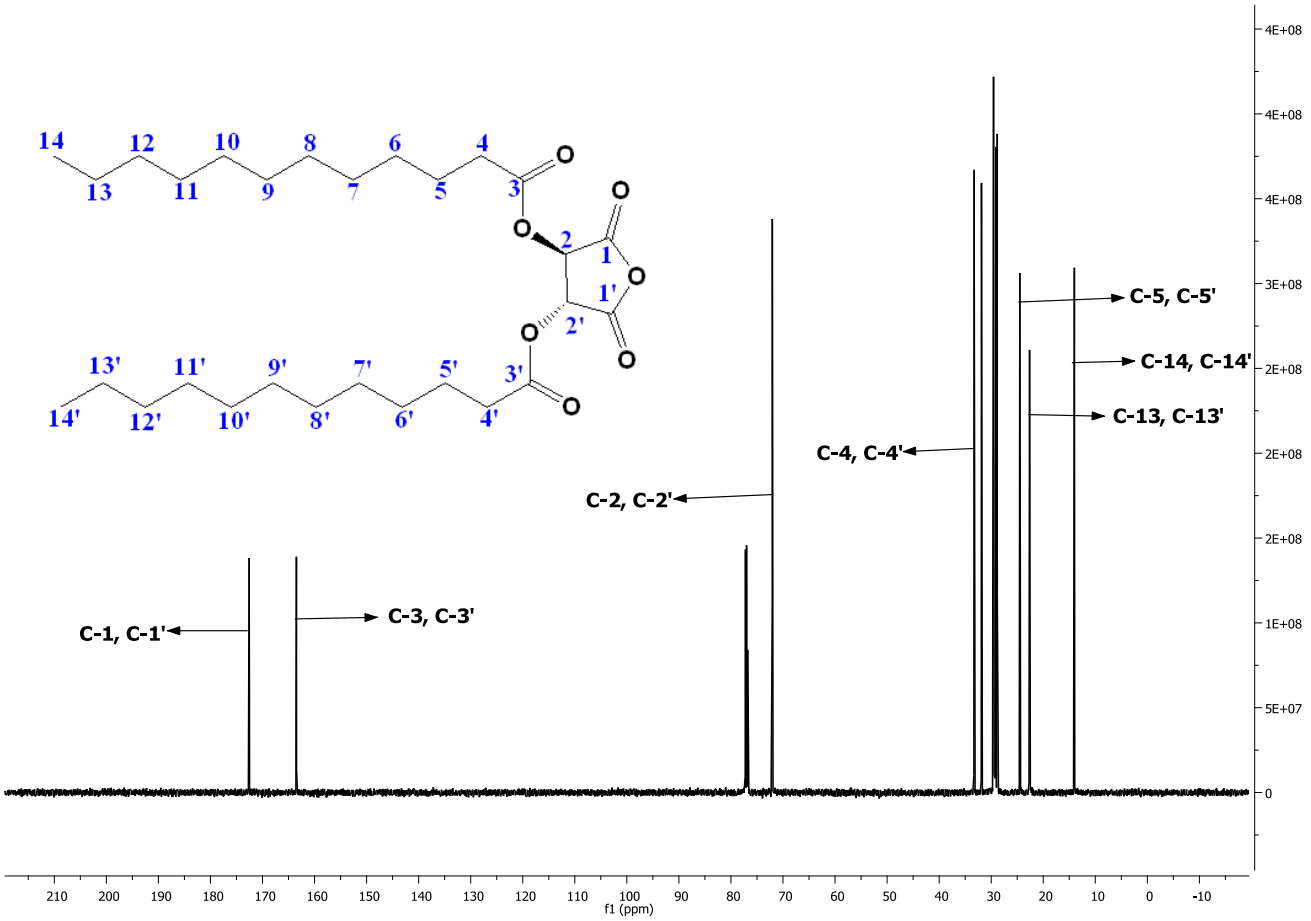

Figure 2, SI: ^{13}C NMR spectrum of compound 1 in $CDCl_3$ recorded at 298 °K.

Synthesis and characterisation of (2*R*,3*R*)-2,3-Bis(dodecanoyloxy)-4-oxo-4-(((2*R*,3*S*,4*S*,5*R*)-3,4,5,6-tetrahydroxytetrahydro-2H-pyran-2-yl)methoxy)butanoic acid (compound 2)

Compound **1** (11 g, 0.022 mol) was added to a solution of *D*-glucose (20 g, 0.11 mol) in anhydrous DMF (150 mL) with stirring under argon and the reaction mixture was cool down to 0 °C, followed by addition of dry pyridine (1.8 mL, 0.022 mol). The reaction was allowed to continued under an argon atmosphere at 0 °C for 2-3 h, followed by an additional 3 days at room temperature. After completion , the reaction mixture was poured into ice-water mixture and then 2 N HCl was added at 0 °C vigorous stirring. The product was collected through extraction in ethyl acetate and the extracetd ethyl acetate layer waswashed four times with brine solution, dried over sodium sulphate and the organic solvent was removed under reduced pressure to obtain the crude product (12.5 g, 83%). The crude product contains a mixture of regioisomers (monoesters) of *D*-glucose (as confirmed by TLC and HPLC-MS and ^{13}C NMR) out of which the 6-substituted monoester compund **2** (~ 80%) was re-precipitated from the mixture in the following manner: the crude mixture was dissolved in a minimum amount of *n*-hexane under reflux and a half volume of acetone was added. The solution was cooled to 0°C in an ice-water bath and then

kept 12 hrs at room temperature. Compound **2** was precipitated from the mixture, filtered and dried to obtain 3.1 g (21%, α/β = 1.8/1) of compound **2** as a mixture of anomers.

^{1}H NMR (400 MHz, acetone-d_6) δ = 5.88 (d, *J* = 2.8 Hz, 1H), 5.85 (d, *J* = 2.6 Hz, 1H), 5.24 (d, *J* = 3.6 Hz, 1H), 4.64 (dd, *J* = 8.1, 4.9 Hz, 1H), 4.46 – 4.32 (m, 1H), 4.09 (ddd, *J* = 9.8, 4.7, 1.9 Hz, 1H), 3.84 (t, *J* = 9.2 Hz, 1H), 3.57 – 3.47 (m, 2H), 2.55 (m, 4H), 1.78 (d, *J* = 7.2 Hz, 4H), 1.66 – 1.27 (m, 32H), 1.03 (t, *J* = 6.7 Hz, 6H) ppm.

^{13}C NMR (101 MHz, acetone-d_6) δ = 173.61, 173.38, 168.12, 167.33, 98.95, 94.49, 78.54, 76.92, 75.64, 75.37, 74.28, 72.31, 72.08, 72.05, 72.01, 70.96, 66.59, 66.41, 34.97, 34.92, 33.29, 30.88, 30.67, 30.35, 30.33, 29.86, 26.21, 26.19, 23.95, 14.94 ppm.
HRMS (ESI$^-$, m/z, [M−H]$^-$) calculated for $C_{34}H_{59}O_{13}$: 675.3956 Found: 675.3951

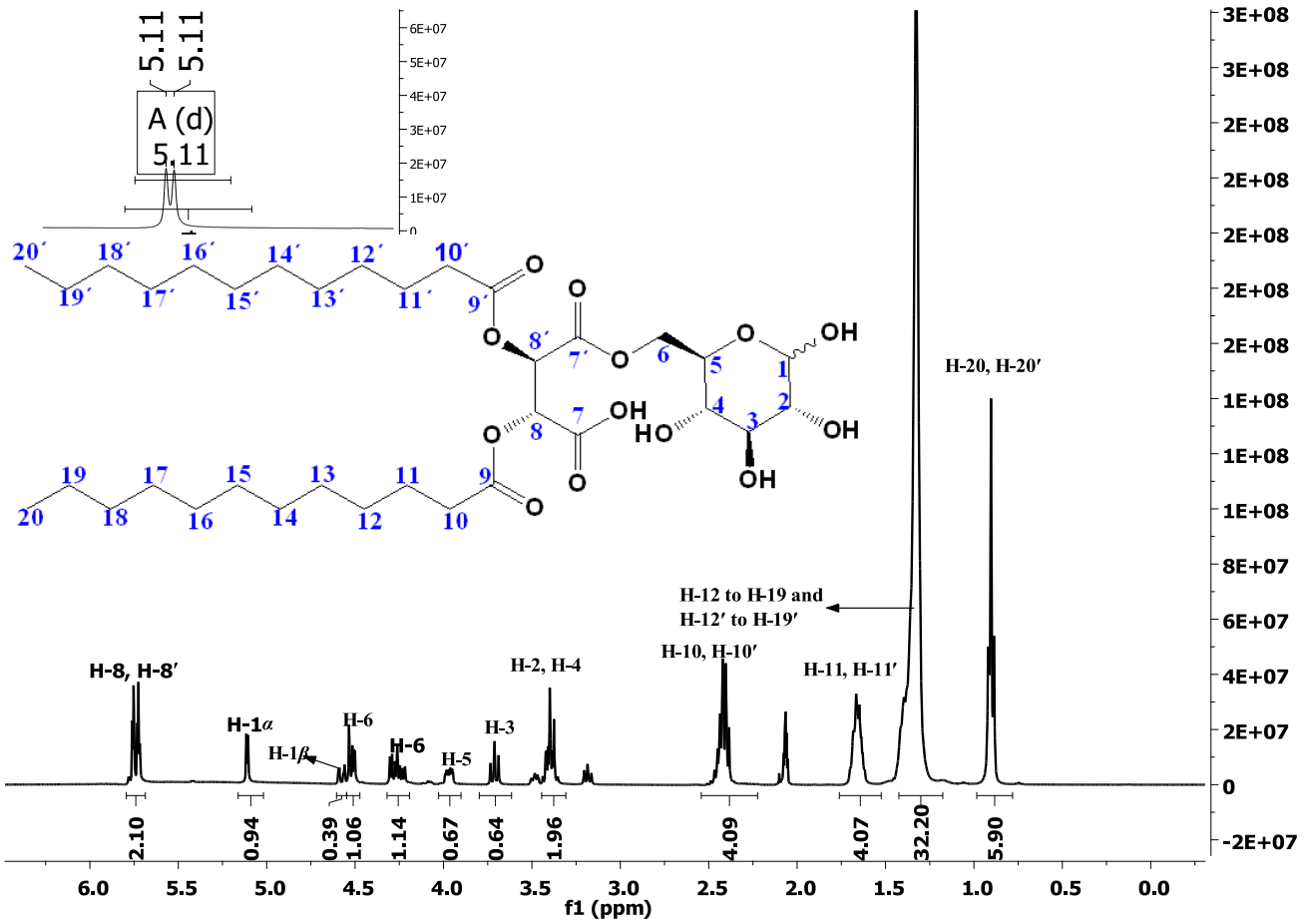

Figure 3, SI: ^{1}H NMR spectrum of compound 2 in acetone-d_6 recorded at 298 °K.

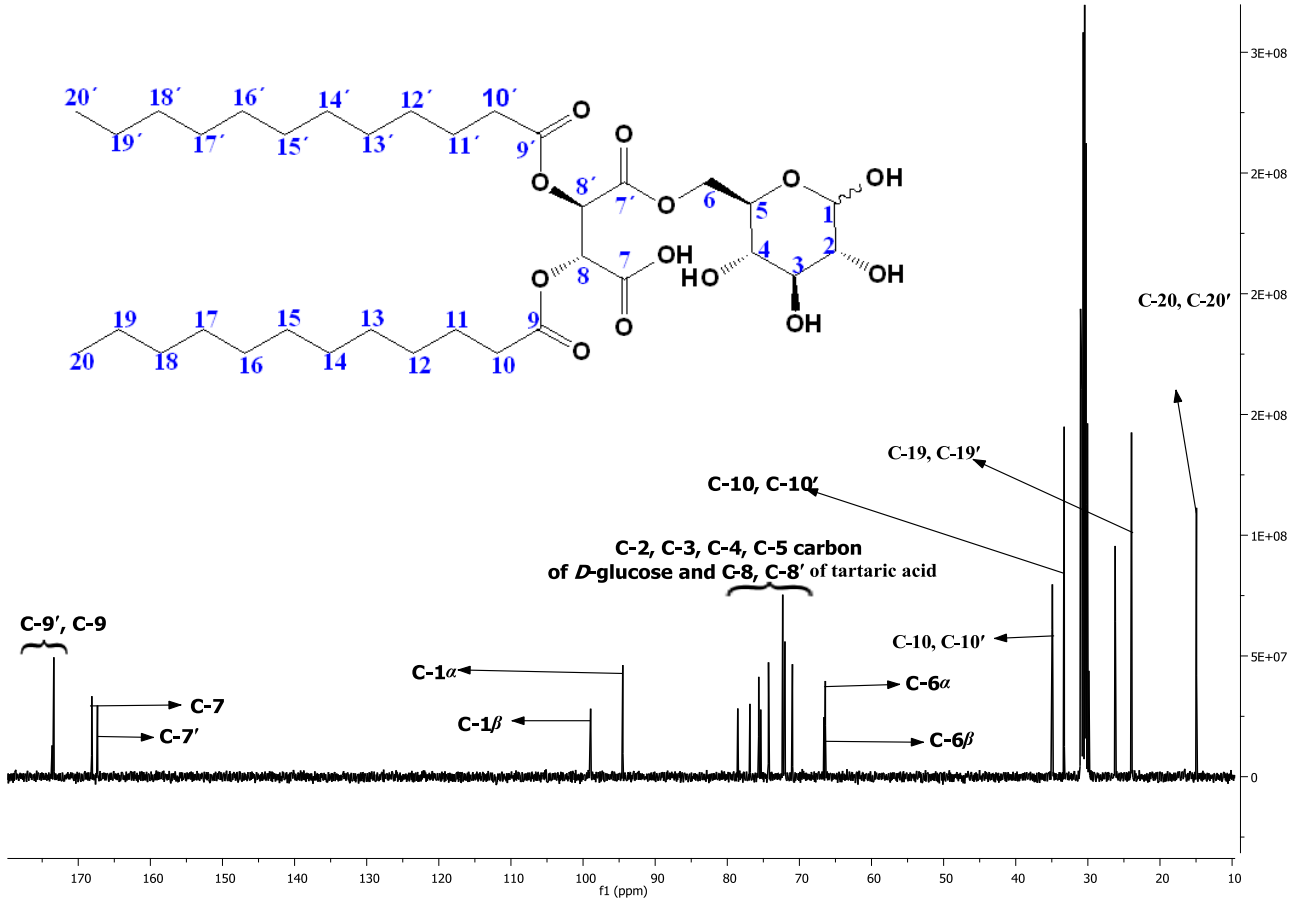

Figure 4, SI: ^{13}C NMR spectrum of compound 2 in acetone-d_6 recorded at 298 °K.

Synthesis and characterisation of amphiphilic carbon quantum dots (CDs, compound 3)

In a teflon film tightened, septum-capped test tube 100 mg of compound **2** and 330 µL DI water were placed and then heated in an oven to 125 °C for 2.5 h. Upon completion of the carbonisation, the reaction mixture was cool to room temperature yielding a brown precipitate which was then re-dispersed in 5 mL of chloroform through vortexing and centrifuged at 7125 rcf for 30 min to remove high-weight precipitate and agglomerated particles. Chloroform was gently evaporated under reduced pressure to obtain a brown solid. The same procedure was repeated with 5 mL acetone, followed by solvent removal under reduced pressure to obtain monodisperse CDs (compound **3**) with a yield of 63 mg.

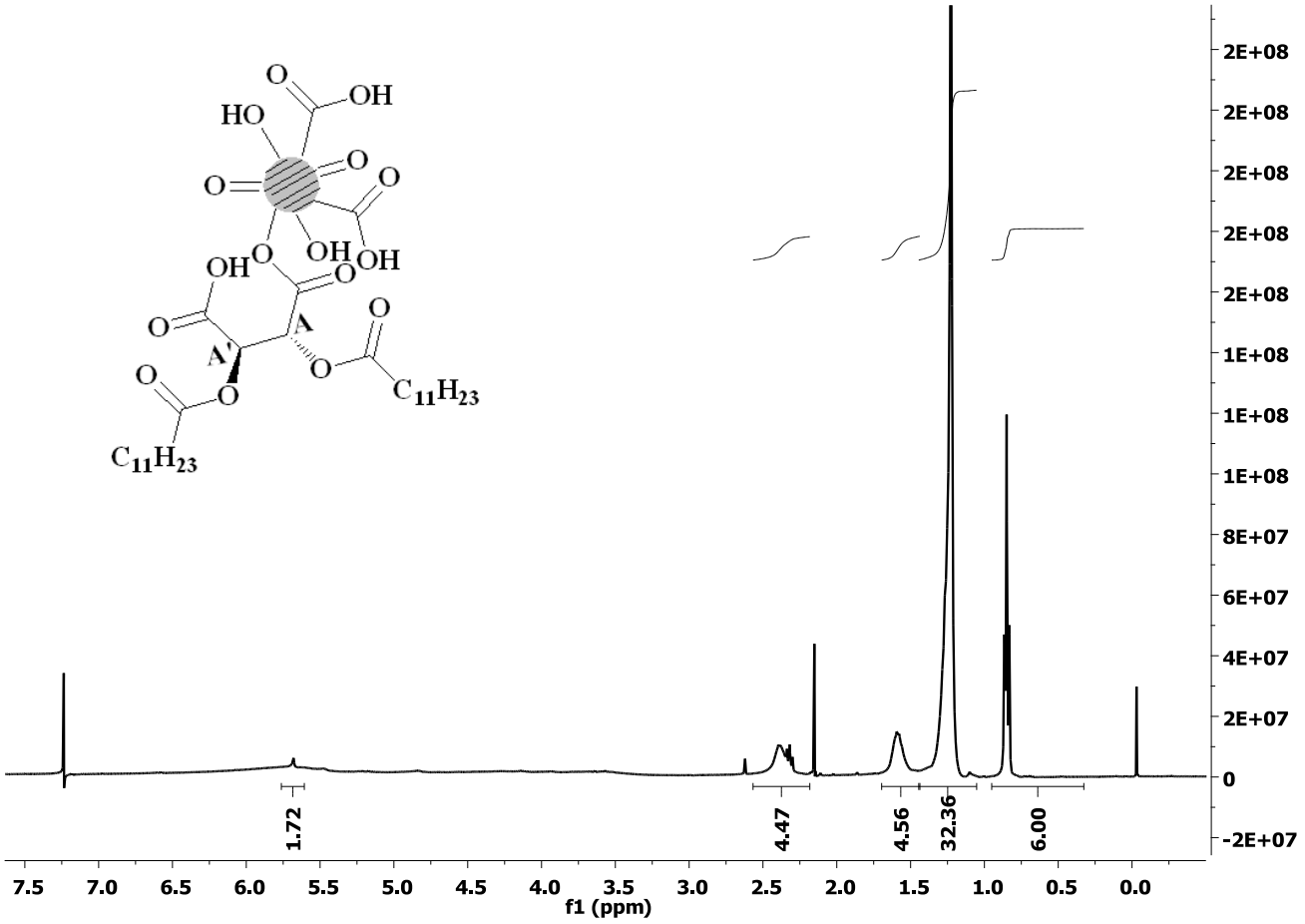

Figure 5, SI: ^{1}H NMR spectrum of CDs (compound 3) in $CDCl_3$ recorded at 298 °K. The ^{1}H NMR spectrum of CDs shows the proton with chemical shifts δ in the range of 2.36 (m, , 4H), 1.59 (m, 4H), 1.23 (m, 32H), 0.85 (t, J = 6.8 Hz, 6H) ppm are the characteristic signal of the lauroyl residue clearly suggesting CDs is coated with hydrocarbon chains (C_{12}). Protons with the chemical shifts of δ = 5.69 – 5.66 (m, 2H) ppm are the H-A and H-A′ proton of the tartaric acid unit.

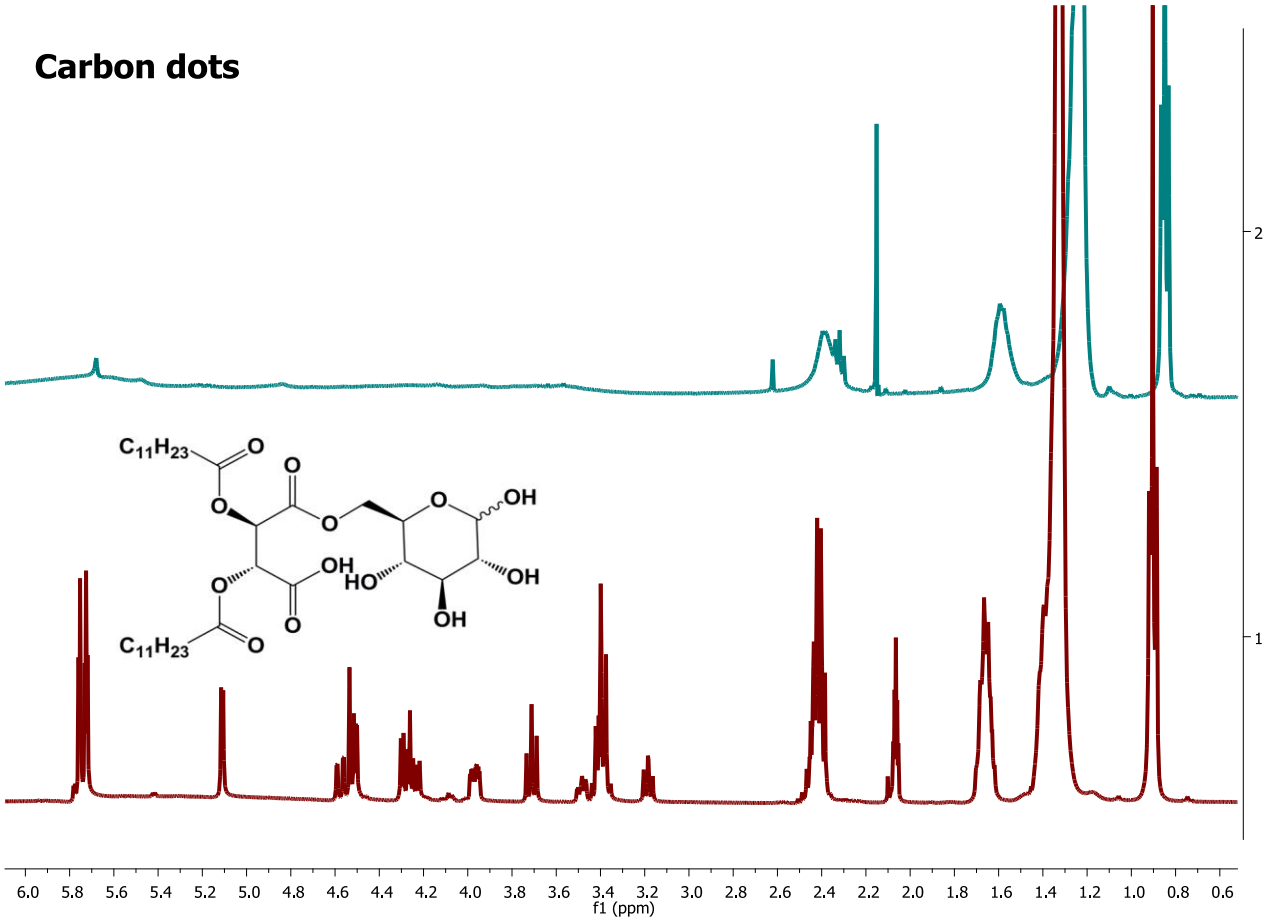

Figure 6, SI: Carbonisation of compound 2 where glucose moiety acts as a source of carbon. ^{1}H NMR spectra of compound **2** (bottom spectrum) and the final product **3** (CDs, top spectrum). In compound **2,** peaks in the range of 5.24–3.57 ppm are ascribed to protons in the carbohydrate ring whereas as in case of the CDs, these signals disappear confirming that carbonisation occurred.

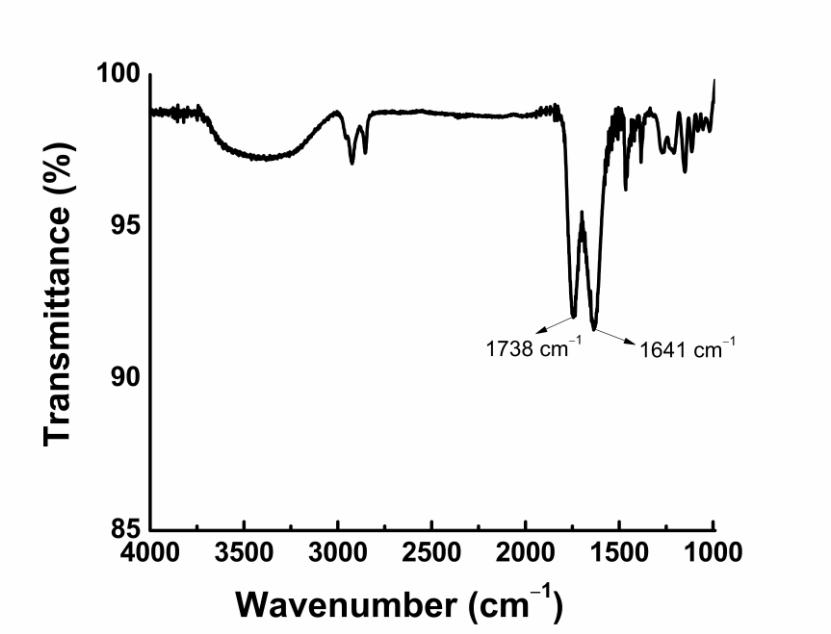

Figure 7, SI: FT-IR spectrum of the as-synthesized CDs (compound 3). The two peaks at 1738 cm^{-1} and 1641 cm^{-1}correspond to the carbonyl and alkyne residues respectively, The absorption band appeared in the range 3000–3600 cm^{-1} is associated with the stretching modes of the hydroxy (–OH) group. The bands at 2850–2925cm^{-1} can be attributed to C−H asymmetric and symmetric stretching vibration, indicating the existence of hydrocarbon chains coated on the CDs surface. The FT-IR spectrum was recorded using a Nicolet-380 FT-IR spectroscopy. FT-IR experiments were carried out by placing a drop of chloroform solution of the sample onto a KBr palette and drying it prior to FT-IR analysis.

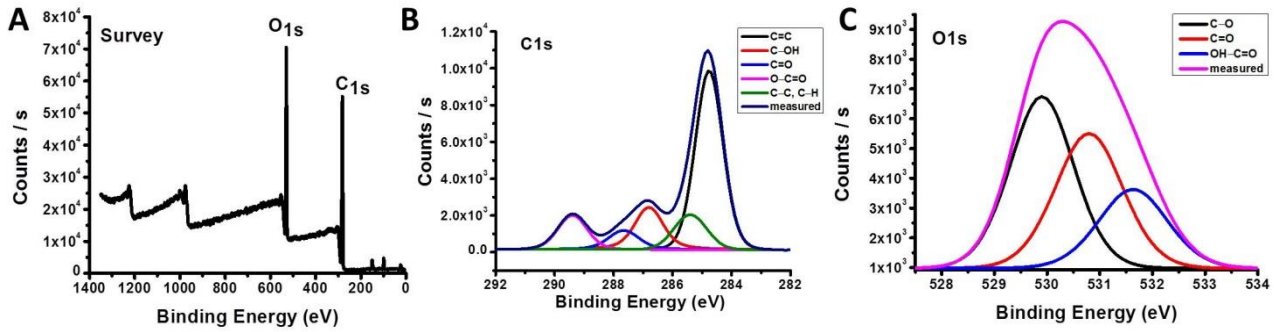

Figure 8, SI: XPS survey spectrum (A) and high-resolution C1s (B) and O1s (C) peaks and the fitting curves of CDs.

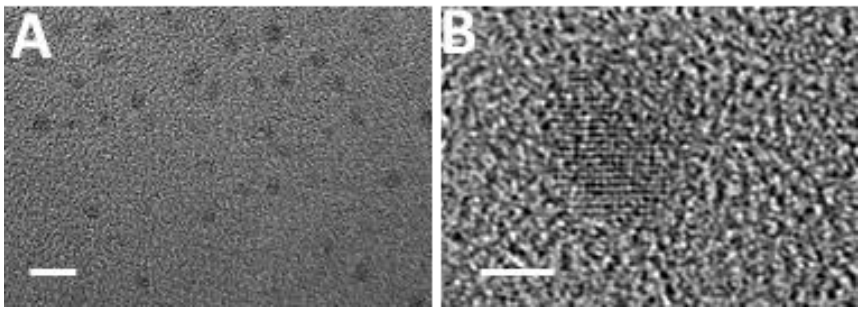

Figure 9, SI: **(A) Transmission electron microscopy (TEM) image of an amphiphilic CD sample. Scale bar is 5** nm; (B) High-resolution TEM (HR-TEM) image showing the crystal planes of an amphiphilic CDs. Scale bar is 2 nm. HRTEM images were recorded on a 200 kV JEOL JEM-2100F. For the HRTEM measurement 0.5 mg of as-synthesised CDs were dissolved in 500 µL chloform and 10 µL of the solution was placed upon an ultrathin carbon film coated-copper grid, dried at room temperature for 2 h and imaged.

Table 1, SI: Correlation between fluorescence emission intensities (excitation 350 nm) of CD-labeled bacteria, and percentage of phosphatydilethanolamine (PE) in the bacterial membrane.[1]

Bacterial strains	Intensity of emission at 350 nm excitation (cells concentration 10^8 CFU/mL)	% of total PE lipid[1,2]
E coli	2.27	> 90%
S. typhimurium	1.94	80%
P. aeruginosa	1.32	60%
B. cereus	1	43%

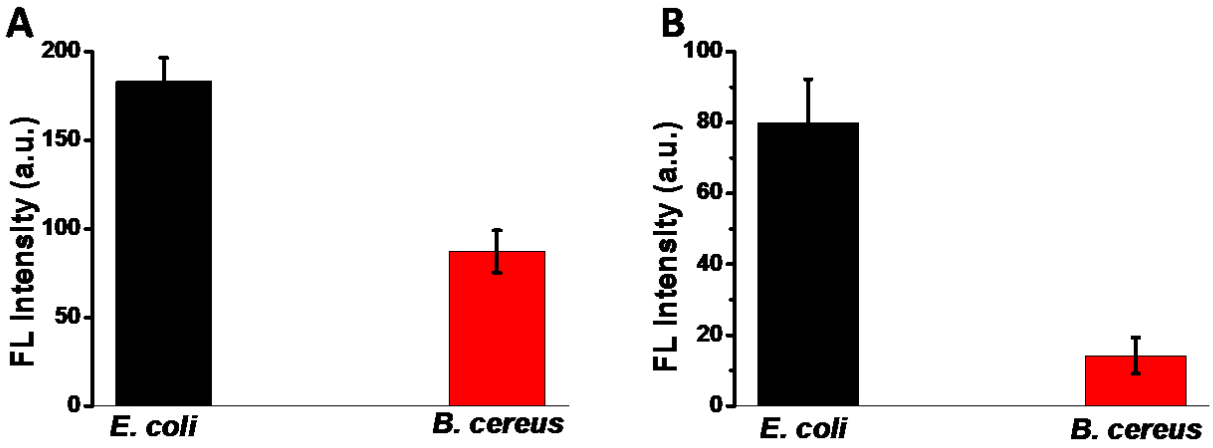

Figure 10, SI: Comparison of fluorescence intensity of *E. Coli* and *B. cereus* in fluorescent microscopy. A. Excitation at 365 nm and 420 nm LP emission filter, **B**. excitation at 540 nm, emission filter 605 nm emission. In each bacterial strain 50 different bacteria was chosen from different place of the cover slide and intensity of each bacteria was calculated individually using ImageJ software and statistical analysis of the fluorescent intensity was done using OriginPro software.

Determination of quantum yield

Using quinine sulfate as a reference, the quantum yield of CDs was found to be 16.5%, 9.4%, and 4.7% in chloroform, hexane, and NaH_2PO_4 buffer, respectively.

Quantum yield of the as-synthesised CDs in a particular solvent was determined by comparing integrated photoluminescence intensity (in the measured range between 400-700 nm) when excited at 375 nm and absorbance value of CDs at 375 nm with integrated photoluminescence intensity (in the measured range between 400-700 nm) when excited at 375 nm and absorbance value at 375 nm of quinine sulfate in 0.1M H_2SO_4 (refractive index (η) of 1.33) according to the following equation:

$$\Phi = \Phi_R \times \frac{I}{I_R} \times \frac{A_R}{A} \times \frac{\eta^2}{\eta_R^2}$$

ϕ=quantum yield

I=Integrated fluorescence intensity

A=Absorbance

n=refractive index

The index R indicates the standard.

REFERENCES

(1) Rana, F. R.; Sultany, C. M.; Blazyk, J. *J. Microbiol. Methods* **1991**, *14*, 41-51.

(2) Epand, R. M.; Epand, R. F. *Mol. BioSyst.* **2009**, *5*, 580-587.